PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference URC054BWO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP2005/001262	International filing date *(day/month/year)* 08.02.2005	Priority date *(day/month/year)* 11.02.2004
International Patent Classification (IPC) or both national classification and IPC C12N1/14		
Applicant UREA CASALE S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.09.2005	21.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Strobel, A Telephone No. +49 89 2399-7362

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP2005/001262

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-14 as originally filed

Claims, Numbers

1-12 filed with telefax on 12.12.2005

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-12
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-12
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: WO 88/06407 A (HANSENS CHR BIO SYST ; NANSEN PETER (DK); GROENVOLD JOERN (DK); HENRIK) 7 September 1988 (1988-09-07)

D2: "A SIMPLIFIED MEDIUM FOR THE PRODUCTION OF HIRSUTELLA THOMPSONII" JOURNAL OF INVERTEBRATE PATHOLOGY, SAN DIEGO, CA, US, vol. 31, no. 1, January 1978 (1978-01), pages 137-139, XP001105514 ISSN: 0022-2011

D3: LIU X Z ET AL: "Nutritional requirements of the nematophagous fungus Hirsutella rhossiliensis" BIOCONTROL SCIENCE AND TECHNOLOGY, vol. 12, no. 3, June 2002 (2002-06), pages 381-393, XP009038514 ISSN: 0958-3157

D4: BASTOS C N: "EFFECT OF TEMPERATURE, PH AND NUTRITION ON GROWTH AND SPORULATION OF TRICHODERMA STROMATICUM SP. NOV., AN ANTAGONIST OF COCOA WITCHES' BROOM PATHOGEN" 2000, SUMMA PHYTOPATHOLOGICA, GRUPO PAULISTA DE FITOPATOLOGIA, PIRACIEABA, ES, PAGE(S) 73-77 , XP001105216 ISSN: 0100-5405

1. **Novelty (Article 33(2) PCT)**
The entire set of claims is considered to be novel over the prior art.

2. **Inventive step of claims 1-4, 10 and 11 (Article 33(3) PCT)**
Said claims are inventive due to the incorporation of the mineral nitrogen source into the claimed culture medium and due to the fact that the claimed method of producing filamentous fungi refers back to the medium of claims 1 and 2.
In fact, the presence, in the culture medium, of an inorganic nitrogen source, increases production of filamentous fungi in terms of cell mass, as shown in example 7. A medium comprising yeast extract or corn steep liquor and at least one organic nitrogen source and a mineral nitrogen source is neither disclosed nor suggested in the prior art.

3. **Inventive step of claims 5-9 and 12 (Article 33(3) PCT)**
Independent claims 5, 6 and 9 concern selection inventions of culture media for filamentous fungi. These media are used in examples 1-6, wherein it is shown

that, seven days after seeding, the dry mass of the cultured fungi is of the order of 9-10 g, with propagules (CFU/l) of 109. The prior art (D1-D4) does not suggest the use of the media of claims 5, 6 or 9 for culturing filamentous fungi nor does it report on comparable growth characteristics associated to the media of the prior art. D4 as particularly relevant prior art reports on growth and conidia formation of fungi cultured in several media (table 2), but the conidia formation remains three orders of magnitude below that of propagule formation in the examples. Propagules and conidia are comparable because conidia are a particular form of propagules.
D1 as particularly relevant prior art describes in example 4 a medium for culturing Arthrobotrys oligospora comprising 78% per dry weight malt extract and 17% corn steep liquor. However, no sporulation was observed and the yield of fungal material of 700 g wet weight.
Thus, claims 5, 6 and 9 contain an inventive step.

4. Additional remarks:

4.1 Unity

Independent claims 1, 5, 6 and 9 newly filed upon entry into the Chapter II phase are not unitary, the common technical feature between these claims being "a culture medium containing an organic C-source and an organic N-source", because claims 5, 6 and 9 are not restricted to media comprising at least one inorganic nitrogen source.
The lack of unity is eventually to be dealt with during the regional phase.

4.2 Support in the description of claim 9

Claim 9 refers to a culture medium comprising "25-20%" malt extract. In the light of the description, page 3, lines 19 to 21, where class 3 culture media are generically disclosed, and to example 5, which is an embodiment of these class 3 culture media generally disclosed on page 3, claim 9 should actually read "25-30%" of malt extract. Claim 9 in its current form thus contains an obvious error.

CLAIMS

1. A culture medium for filamentary fungi comprising at least one carbon source chosen from the group consisting of molasses, malt extract and sucrose and at least one organic nitrogen source chosen from yeast extract and corn steep liquor, the culture medium further comprising a mineral nitrogen source.

2. A culture medium according to claim 1, wherein said at least one carbon source constitutes 70 to 85% by weight of the dry weight of the culture medium and said at least one organic nitrogen source constitutes 15 to 30% by weight of the dry weight of the culture medium.

3. A culture medium according to claim 1 or 2 wherein said mineral nitrogen source is contained in an amount no greater than 10% by weight of the dry weight of the culture medium and preferably between 5 and 8% by weight.

4. A culture medium according to claim 3 wherein said mineral nitrogen source consists of ammonium nitrates or salts.

5. A culture medium for filamentary fungi consisting of 75-85% malt extract and 15-25% yeast extract, wherein said percentages are by weight of the dry weight of said culture medium.

6. A culture medium for filamentary fungi comprising 60-65% molasses, 10-15% sucrose, 10-15% corn steep liquor and 10-15% yeast extract.

7. A culture medium according to claim 6, further comprising 5 to 8% of a mineral nitrogen source.

8. A culture medium according to claim 7, wherein said mineral nitrogen source consists of diammonium hydrogen phosphate.

9. A culture medium for filamentary fungi containing, in percentage by weight of the dry weight of said medium, 25-20% malt extract, 40-45% molasses and 25-30% corn steep liquor.

10. A method for producing filamentary fungi, in particular nematophagus fungi, on an industrial scale, comprising the step of seeding conidia of said fungi in a culture medium according to any one of claims 1 and 2 and keeping said culture medium at a temperature of 23-30°C for a time of 5-10 days to determine the reproduction and growth of the fungi, wherein the mineral nitrogen source of said culture medium is gradually added in small amounts, preferably from the fourth day after the seeding of said conidia

11. A method according to claim 11, wherein said mineral nitrogen source consists of ammonium nitrates and salts and it is added in a total amount of no more than 10% of the dry weight of said culture medium and preferably in an amount between 5 and 8% of the dry weight of said culture medium.

12. A method for producing filamentary fungi, in particular

nematophagus fungi, on an industrial scale, comprising the step of seeding conidia of said fungi in a culture medium according to any one of claims 5, 6 and 9 and keeping said culture medium at a temperature of 23-30°C for a time of 5-10 days to determine the reproduction and growth of the fungi